(d) simultaneously incubating said mini-proinsulin compound with trypsin and carboxypeptidase B at a pH of about 6.8 to produce insulin, under conditions where no crystals are formed; followed by

(e) precipitating the insulin.

REMARKS

Applicants request entry of this amendment, reconsideration, and timely issuance of a Notice of Allowance. The remarks and amendments place the application in condition for allowance or better form for appeal. In addition, no further searches or new issues are raised by the amendments.

Claims 21-23 and 25-27 are pending.

Applicants have amended claims 21, 22, 25, and 26. Support for the amendments can be found in the disclosure as a whole and, for example, at page 15, lines 21-35, which describe the cleavage step in solution. The appropriate conditions for incubating the mini-proinsulin compound with an enzyme where no crystals are formed during the cleavage can, therefore, be gleaned from the disclosure in the specification. Accordingly, the claimed methods refer to, in one aspect, an enzymatic cleavage step that occurs while the resulting mono-Arg-insulin or insuiin is in solution and not present as a crystal. Thus, no new matter enters by these amendments.

Claims 21-23 and 25-27 stand rejected under 35 U.S.C. § 103 as allegedly being unpatentable over Markussen et al. ('212 patent) or Markussen et al. (EPO) either in view of

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Goeddel et al., Mai et al., Grau et al. ('684 patent), and Grau ('332 patent) essentially as applied in a prior Office Action (Paper No. 19).

In Paper No. 29, the immediately preceding Action, the Office stated that "[t]he limitation of conditions where no crystals are formed would be met as the prior art does not absolutely require crystallization. . . ." (Paper No. 29 at pages 4 and 5.) However, it is the Office's burden to present a *prima facie* case of obviousness wherein the "prior art" suggests not only a combination to arrive at applicants' claimed invention, but also a reasonable expectation of success. In re Vaeck, 20 U.S.P.Q.2d 1438, 1442 (Fed. Cir. 1991). Here, the contents of the cited documents, along with other available documents, do not support a combination as made by the Office.

Markussen '212 and Markussen (EPO) discuss methods for producing "insulin precursors." (Markussen '212, at col. 2, lines 33-39, for example.) The "insulin precursors" of Markussen differ from the recited mono-Arg-insulin and insulin in that they have not been properly converted into the two chain form of insulin. For example, the "natural" single chain precursor to insulin is the single chain B-C-A polypeptide, where the C chain is removed by proteolytic cleavage to convert the polypeptide into a two chain insulin molecule. (See Figure 29-10 in the accompanying excerpt of Zubay's "Biochemistry.")

The required "in vitro conversion" to arrive at a process producing mature human insulin, as discussed in the Markussen documents, refers only to a process involving L-threonine esters. (Markussen '212, at col. 5, lines 3-11; and Examples 14-18 at col. 18, line 25, through col. 19, line 33.) Markussen's use of this method supports a conclusion that those skilled in the art would

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not have expected trypsin to cleave at the C-terminus of a bridging Arg residue in the single chain "precursor" (the Arg of the recited formula B(1-30)-Arg-A(1-21)) to ultimately generate the two chain, mature form of insulin. Why else would one go through the additional steps involved in the L-threonine ester and not discuss or even mention other more direct methods such as cleavage with trypsin? Accordingly, applicants' invention of methods wherein trypsin can be used in cleaving the single chain "precursor" into the final insulin or mono-Arg-insulin is not taught or suggested by the Markussen documents. In fact the Markussen documents teach different methods that suggest against cleavage with tryspin.

Furthermore, applicants have argued that Thim et al. indicate that trypsin cannot cleave a miniproinsulin with a single Arg bridge between the B and A chains. (Amendment filed November 6, 1995, at page 11.) The Office's response at pages 5-6 of the Action appears to confuse the cleavage of "additional protein" in the optional fusion protein construct discussed in Markussen '212 with the enzymatic cleavage that produces insulin from a single chain precursor. In other words, Markussen '212 discusses such cleavage of a fusion protein at the N terminus of the B chain rather than cleavage of the single chain B(1-29)-X_n-Y-A(1-21) "insulin precursor" to generate the mature, two chain insulin. (See Markussen '212, at col 3, lines 36-41.) For example, "[t]he insulin precursors may be expressed with additional protein proceeding the insulin precursor." (Markussen '212, at col. 3, lines 52-53; see also col. 4, lines 19-24 discussing N-terminal "superfluous amino acid sequence.") The discussion the Office cites from Markussen '212 (col. 4, lines 26-29) relates to that cleavage of an insulin precursor from "an amino acid sequence linked to the B(1-29)-chain" or "additional N-terminal amino acid-sequence to be

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removed." (Paper No. 32 at page 5.) It does not, however, relate to the enzymatic cleavage step involving the generation of insulin from a single chain precursor. Thus, the Office's comments do not rebut applicants' showing that Thim et al. teach away from the combination the Office proposes since one skilled in the art would not have expected trypsin to cleave at a single Arg residue in the applicants' recited formula B(1-30)-Arg-A(1-21). This enzymatic cleavage results in a two chain insulin or mono-Arg-insulin product according to the claimed invention.

In addition, the Markussen documents do not discuss preparing a mono-Arg-insulin as claimed herein. The Office relies on Grau '332 for discussions of mono-Arg-insulin. (Paper No. 32 at page 3.)

In Grau '332, the enzymatic cleavage step of the single chain "intermediate" occurs close to the isoelectric point of insulin in the presence of aromatics. (Grau '332, at col. 3, lines 12-34, and Example 1 at col. 3, line 56, through col. 4, line 29.) As a result, there is a direct precipitation of the cleavage product into crystalline form. (Grau '332, at col. 2, lines 2-10.) That precipitation results in the insulin being resistant to further digestion, which is an advantage stressed as being important in Grau '332. (Grau '332, at col. 2, lines 10-12.) It is not the structure of the resulting mono-Arg-insulin that *per se* renders the insulin stable in the method discussed in Grau '332, as alleged by the Office, since Grau '332 stresses the importance of the crystalline form. (Paper No. 32 at page 5.) In fact, to change the crystallization step of Grau '332 in order to arrive at methods as claimed would remove the entire benefit of crystallization in the method discussed by Grau '332. For example, the resulting insulin derivatives or insulin of Grau '332 would no longer precipitate.

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As noted above, applicants now claim methods wherein the mono-Arg-insulin or insulin is produced through an enzymatic cleavage step that occurs under conditions where no crystals are formed. Thus, the resulting mono-Arg-insulin or insulin remains in solution until the following precipitation step. Since Grau '332 asserts a benefit in the immediate precipitation of insulin, to remove that benefit is a teaching contrary to Grau '332. Thus, Grau '332 teaches away from a method as applicants now claim and Grau '332 cannot motivate one skilled in the art to arrive at a method as claimed.

In summary, one skilled in the art would not have been led to use a compound, such as a single Arg derivative discussed in Grau '332, in a process that defeats the alleged benefit of the Grau '332 process. Furthermore, the methods discussed in the Markussen documents do not suggest ignoring the alleged benefits of the processes involved with the compounds discussed in Grau '332. And, since the Markussen documents do not teach or suggest an enzymatic cleavage method wherein a single Arg "precursor" is used, there is no motivation to combine the teachings of Grau '332 and the Markussen documents as the Office has done.

The Goeddel, Mai, and Grau '684 documents do not address or remedy the deficiencies in the combination discussed above. None of these documents discuss methods to prepare insulin or mono-Arg-insulin from the recited formula B(1-30)-Arg-A(1-21). Thus, none of these documents could have led one skilled in the art to the claimed invention in combination with the Markussen documents or Grau '332.

For these reasons, applicants traverse the section 103 rejection, and respectfully request reconsideration and withdrawal of it.

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If there are any other fees due in connection with the filing of this paper, please charge such fees to our Deposit Account No. 06-0916. If an extension of time is required under 37 C.F.R. § 1.36 and not accounted for above, such an extension is respectfully requested and the fee should be charged to Deposit Account No. 06-0916.

Respectfully Submitted,

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